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(54) Title: ANTI-VIRAL COMPOUNDS

(57) Abstract: Compounds of Formula I wherein R_1 is CH_2CO_2K, CH_2CO_2H or CH_2CONH_2 and R_2 is where Hal is a halogen, preferably F or Br, and L is H or a halogen, preferably Br and Formula II are described. The compounds are useful as pharmaceutical compositions in the treatment of AIDS.

Title

Anti-viral compounds

Field of the Invention

The present invention relates to compounds having biological activity and to processes for the preparation thereof. The invention is particularly directed to compounds having anti-viral, particularly anti-HIV activity.

Background to the Invention

The virus that causes AIDS, the human immunodeficiency virus HIV is believed to be one of the major threats to human life and health worldwide. Even back in 1988 an article in Scientific American by J.M. Mann, J. Chin, P. Piot and T. Quinn estimated that more than a quarter of a million AIDS cases had occurred in the U.S.A. up to then and that 5-10 million people were infected worldwide. An article in the same magazine ten years later "Defeating Aids: What will it take? (July 1998 page 62) revealed that worldwide 40 million people had contracted HIV and almost 12 million had died leaving over 8 million orphans. During 1997 alone nearly 6 million people acquired HIV and some 2.3 million perished including 460,000 children.

Although 90% of HIV infected people live in developing countries well over 90% of money for care and prevention is spent in industrial countries. The very expensive triple therapy drugs (over US$10,000-$15,000 per person per year) are well beyond the reach of individuals in developing countries in sub Saharan Africa and Asia. In 1999 alone, 300,000 people died in Ethiopia from AIDS far exceeding deaths from famine (12 April 2000, The Irish Examiner). Up to a quarter of South Africa's non-whites currently face death from AIDS in the next ten years (11 May 2000, The Irish Examiner, by G. Dyer). There is thus a desperate need for cheap, easily made and efficient anti-HIV agents for the developing world.

The HIV has been studied more intensively than any other virus and we now have a general picture of how the genes and proteins in the HIV virus particle operate, although we don't have a clear understanding of what controls the replication and how it destroys the human immune system. There are in fact many strains of HIV. The two

main ones are HIV-1 and HIV-2. HIV-2 is prevalent in West Africa and produces a less severe disease than does HIV-1 the most common form elsewhere.

The life cycle of the virus is described below in some detail since for a drug to be effective it has to interfere with at least one stage of its life cycle. The HIV virus particle is roughly spherical shaped and is about a thousandth of a millimetre across. Its outer membrane consists of lipid molecules which posess many viral protein spikes projecting outwards. Each spike is thought to consist of four molecules of glycoprotein gp120 with the same number of glycoprotein gp41 molecules embedded in the membrane itself. These envelope proteins come into play when HIV binds and then enters target cells. Gp120 can bind tightly to CD4 proteins sited in the membranes of immune system cells especially T lymphocytes also called T cells. This is the first stage of the infection which is followed by fusion of the virus and T cell membrane, a process governed by the gp41 envelope protein. The result is that the contents of the virus core are thus freed to enter the cell. The virus core is surrounded by matrix protein called p17 and is itself in the shape of a hollow cone made of another protein p24 containing the genetic material of the virus.

Being a retrovirus this genetic material is in the form of RNA (ribonucleic acid) consisting of two RNA strands. These are in turn attached to molecules of an enzyme, reverse transcriptase, which transcribes the viral RNA into DNA once virus has entered the cell. Coexisting with RNA are an integrase, a protease, a ribonuclease and other enzymes. Once in the cell the viral RNA is converted to DNA which then enters the cell nucleus. The next step is integration of viral DNA into host chromosomes. This is followed by cell proteins binding to DNA initiating transcription. Short RNA molecules then leave the nucleus and make viral regulatory proteins followed by medium length and long RNA which generate structural and enzymatic proteins. These assemble to form new viruses (replication-viral budding) (1).

Prior to 1991 the only drug available to combat HIV/AIDS was Glaxo-Wellcome's AZT (zidovudine) a nucleoside analogue which works by binding to the reverse transcriptase enzyme thereby inhibiting viral replication. Unfortunately, long term use led to the virus developing resistance against the drug by mutation. New drugs in the same class were subsequently developed including 3TC (lamivudine) (Glaxo-

Wellcome), ddc (zalcitabine) (Roche), ddl (didanosine)(Bristol-Myers Squibb), d4T (stavudine) (Bristol-Myers Squibb) and recently abacavir (Glaxo-Wellcome).

1996 saw the introduction of a new class of drugs which acted at a different (and later) stage in the HIV virus' life cycle by blocking the action of the protease enzyme during viral replication. Furthermore, use of one of these with two of the class above (reverse transcriptase) gave viral loads in the blood being reduced by up to 4 log units or by a factor of ten thousand. Use of one drug alone reduces viral load by up to 2 log units or by a factor of one hundred. An effective example of this so called triple therapy would be use of AZT and 3TC (reverse transcriptase inhibitors) and indinavir (Merck Sharp and Dohme) or nelfinavir (Agouron) (protease inhibitors). Other protease inhibitors include saquinavir (Roche), ritanovir (Abbott laboratories) and amprenavir (Glaxo-Wellcome). In general, effective therapies employ two reverse transcriptase inhibitors together with one protease inhibitor.

1996 also saw the introduction of another new class of drugs known as non-nucleoside reverse transcriptase inhibitors, the first being nevirapine (Boehringer Ingelheim) followed by delavirdine (Pharmacia Upjohn) in 1997 then efavirenz (Du Pont) in 1998.

New effective therapies also capable of reducing viral loads by up to 4 log units or by a factor of 10,000 employ a combination of nucleoside and non-nucleoside reverse transcriptase inhibitors using a total of at least three drugs.

The cost of any triple therapy per patient per year is £10,000 -£15,000. (2)

The following table gives an overview of current AIDS drugs, their type or class, effectiveness in reducing viral load, total amount of drug given to patient each day in number of doses, side-effects, time for viral drug resistance to develop when used alone, and approximate cost per patient per year. (2).

The first mentioned nucleoside reverse transcriptase enzyme inhibitor zidovudine (AZT) when used by itself has subsequently been shown to provide no benefits in treating HIV-infected individuals (3) although it is effective reducing transmission from mother to baby (4).

However, it can be effective when used in conjunction with other AIDS drugs such as 3TC, another nucleoside reverse transcriptase enzyme inhibitor (5).

Additionally, the HIV virus develops viral drug resistance against AZT rather quickly (5-6 months) when used alone and even more rapidly (1 and a half months) against 3TC when used alone (2). All nucleoside reverse transcriptase enzyme inhibitors can cause serious side effects ranging from myopathy to peripheral neuropathy (nerve damage). The most recent drug abacavir's side effects can be life-threatening so treatment with this drug is immediately stopped at the first signs of any adverse reactions. Also ddc is a very toxic drug. Reduction in viral loads by drugs used on their own are only moderate 50-90% and their cost is quite high (£1,200-£10,000 per patient per year) (2).

The relatively recently developed non-nucleoside reverse transcriptase enzyme inhibitor AIDS drugs can cause severe skin reaction in patients and the HIV virus can develop viral drug resistance against them very quickly in 2 months in monotherapy (one drug). In addition, cross viral drug resistance has been noted using this class of drugs. In this case drug resistance against one drug in the class can cause drug resistance against another drug of the same class (2). Again used by themselves they only reduced viral load in patients by 50-90% and are relatively expensive (£1800-£2400 per person per year) (2).

The new protease enzyme inhibitors have to be given to patients in relatively large amounts (1250-2400mg per day) and can give serious side effects ranging from kidney stones to hepatitis and after prolonged use patients exhibit raised levels of cholesterol and triglycerides and can cause diabetes and abnormal distribution of body fat. In addition they are expensive (£4000-£7000 per person per year) (2). They are also generally poorly absorbed and have poor bioavailability which could well be related to their low water solubility (6), (Protease Inhibitors in Patients with HIV disease by M.Barry, S. Gibbons, D. Back and F. Mulcahy in Clinical Pharmacokinetics March 32 (3) 1997 p194) and can interact with other protease enzyme inhibitors and nucleoside/non-nucleoside enzyme inhibitors in combination therapy, giving rise to a very strict order of oral dosing which must be adhered to by the patient (7) (Pharmacokinetics and Potential Interactions amongst Antiretroviral Agents used to treat patients with HIV infection by M. Barry, F. Mulcahy, C. Merry, S. Gibbons and D. Back, Clinical Pharmacokinetics, April 36(4) 1997 p289).

MARKETPLACE COMPARISON						
DRUG	TYPE	REDUCTION IN VIRAL LOAD	TOTAL AMOUNT DRUG/DAY in (x) doses	SIDE EFFECTS	VIRAL DRUG RESISTANCE (MTHS)	COST/ PATIENT/ YEAR (PUNTS) COMPANY
Zidovudine (AZT)	nucleoside reverse transcriptase inhibitor	50-90%	600mg (2)	myelosupression, myopathy, nausea, headache, anaemia	5-6	£7,000-£10,000 Glaxo-Wellcome
Lamivudine (3TC)	nucleoside reverse transcriptase enzyme inhibitor	50-90%	300mg(2)	gastrointestinal disturbances, hair loss, myelosuppression, exacerbation of peripheral neuropathy	1½	£7,000 Glaxo-Wellcome
Stavudine (d4T)	nucleoside reverse transcriptase enzyme inhibitor	50-90%	40mg(2)	peripheral neuropathy	greater than 6	£1,800 Bristol Myers Squibb
Didanosine (ddl)	nucleoside reverse transcriptase enzyme inhibitor	50-90%	300-400mg (1) (at night)	peripheral neuropathy, nausea vomiting, pancreatis	greater than 6	£2,000 Bristol Myers Squibb
Zalcitabine (ddc)	nucleoside reverse transcriptase enzyme inhibitor	50-90%	0.75mg (1) (with meals)	very severe peripheral neuritis	greater than 6	£1,200 Roche
Abacavir	nucleoside reverse transcriptase enzyme inhibitor	50-90%	300mg(2)	any reaction can be life-threatening always stopped immediately	-	£2,400 Glaxo-Wellcome

DRUG	TYPE	REDUCTION IN VIRAL LOAD	TOTAL AMOUNT DRUG/DAY in (x) doses	SIDE EFFECTS	VIRAL DRUG RESISTANCE (MTHS)	COST/ PATIENT/ YEAR (PUNTS) COMPANY
Nevirapine	non-nucleoside reverse transcriptase enzyme inhibitor	50-90%	200mg (2)	skin reaction	2	£1,800 Boehringer Ingelheim
Delaviridine	non-nucleoside reverse transcriptase enzyme inhibitor	50-90%	600mg (3) many tablets	skin reaction	2	£1,800 Pharmacia-Upjohn (Agouron)
Efavirenz	non-nucleoside reverse transcriptase enzyme inhibitor	50-90%	600mg (1)	skin reaction	2	£2,400 Dupont
Indinavir	protease enzyme inhibitor	99%	2400mg (3)	hyperbilrubinaemia, nephrolthiasis, nausea, kidney stones, dizziness	6	£5,000-£7,000 Merck Sharp & Dohme
Ritonavir (not used by itself)	protease enzyme inhibitor	99%	1800mg (2)	diarrhoea nausea, vomiting, hepatitis, headache	6	£5,000-£7,000 Abott Laboratories
Saquinavir	protease enzyme inhibitor	99%	1800mg (2)	loose stools, nausea, headache	6	£5,000-£7,000 Roche

DRUG	TYPE	REDUCTION IN VIRAL LOAD	TOTAL AMOUNT DRUG/DAY in (x) doses	SIDE EFFECTS	VIRAL DRUG RESISTANCE (MTHS)	COST/ PATIENT/ YEAR (PUNTS) COMPANY
Nelfinavir (Viracept)	protease enzyme inhibitor	99%	1250mg (2) a lot of tablets total 10	diarrohea, nausea & vomiting	6	£4,000-£5,000 Agouron (Roche)
Amprenavir (can be used with Ritonavir)	protease enzyme inhibitor	99%	a lot of tablets	severe rash	-	£7,000 Glaxo-Wellcome
All protease enzyme inhibitors raise patient's cholesterol, triglyceride levels and can cause diabetes, kidney stones and abnormal distribution of body fat after prolonged use.						

The concentration at which an HIV-1 drug is effective is designated EC_{50} µm which represents when the number of cells protected from HIV injection is half the total. The antigen Agp120 assay - the virus related antigen - is related to the number of virus particles produced by measuring glycoprotein gp 120 in infected cell cultures. The concentration of the drug which reduces cell growth by 50% is designated TC_{50}µM.

Of course the lower the EC_{50} concentration the better but the real criterion of effectiveness in in vitro testing on cell cultures is the Therapeutic index which is the TC_{50}/EC_{50} ratio. The therapeutic index is selected so as not to damage healthy cells. Thus AZT has an EC_{50} of ca 0.016 µM with a TC_{50}>1000 µM. This results in a therapeutic index of >1000/0.016 => 62,500. This figure serves as a benchmark against which new potential drugs can be measured. Of course human beings and animals are more than a collection of cells and in spite of the high Therapeutic Index, AZT is quite toxic, giving rise to nerve damage and anaemia among other things (2). Nevertheless, such tests on cell cultures indicate what is a potential anti-HIV drug.

Other factors relevant to the usefulness of an anti-HIV drug are physical properties such as water-solubility for drug absorption by the patient and stability of the

compound after oral intake. Thus the potentially useful drug, the anionic polysaccharide, dextran sulphate is poorly absorbed orally and degrades after oral intake before entry into the plasma (8). Another important factor is the ease of synthesis of the drug and hence drug cost which is relatively high for AZT and most other drugs produced to date which are potentially useful in combating AIDS.

International Publication No. WO9403164 decribes compounds having biological activity, particularly sulfonate based calixarenes, having anti-HIV activity. The present application relates to compounds selected from the general group of compounds disclosed in international application no. PCT/IE 95/00008 having especially surprising activity. This application relates in particular to cyclic tetrameric pyrogallol-aldehyde derivatives and to calixarene derivatives which are useful in the treatment of AIDS.

As mentioned previously, a high therapeutic index is required for an anti-HIV drug to be effective. In particular international application no. PCT/IE95/00008 discloses (AC-3 (Example 40 in PCT/IE95/00008)) the potassium acetate of p-nitrocalix-4-arene. This compound has a relatively low therapeutic index of >375 (in vitro) as compared to AZT which has a therapeutic index of >62,500. Such a comparison of values teaches away from the use of this particular compound in pharmaceutical compositions for the treatment of patients with HIV-1. Surprisingly, however, this compound appears to be particularly effective as an anti-AIDS drug.

The present application also relates to the use of this compound in a pharmaceutical composition for the treatment of HIV-1.

There is a need for an anti-HIV drug which brings about a reduction in viral load but without causing the development of viral drug resistance and problems of toxicity. In short, a drug is needed which when given orally gives rise to at least a M.I.C. (Minimum inhibitory concentration) of drug in the blood against HIV but at a low enough concentration so as not to give rise to adverse side effects in the patient.

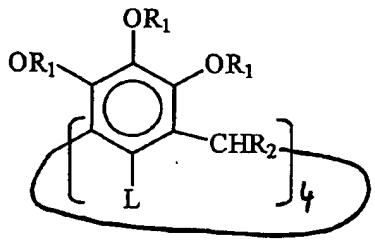
Object of the Invention

It is an object of the present invention to provide novel and easily synthesised compounds having biological activity, particularly anti-HIV activity, particularly against HIV-1.

It is a further object of the invention to provide compounds having a low EC_{50} or MIC in patients blood (plasma) concentration which exhibit reduced and preferably little or no side effects, and bring about a reduction in viral load but without causing the development of viral drug resistance and pharmaceutical compositions thereof.

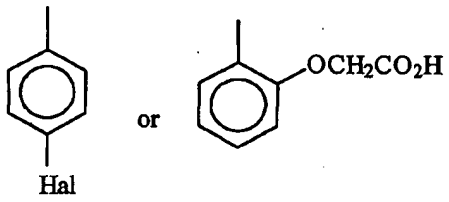
Summary of the Invention

The invention provides compounds of formula I



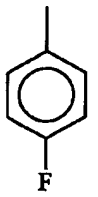
Formula I

wherein R_1 is CH_2CO_2K, CH_2CO_2H or CH_2CONH_2 and R_2 is

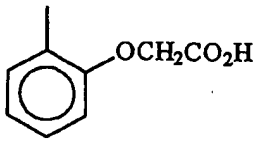


where Hal is a halogen, preferably F or Br, and L is H or a halogen, preferably Br.

In a particular embodiment the invention provides compounds of formula I wherein wherein R_1 is CH_2CO_2K or CH_2CO_2H and R_2 is



or

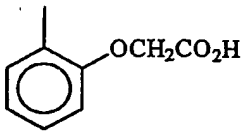


and L is H or a halogen, preferably Br.

In another preferred embodiment the invention provides a compound wherein R_1 is CH_2CO_2K, R_2 is

and L is Br.

Further preferably the invention provides a compound wherein R_1 is CH_2CO_2H, R_2 is

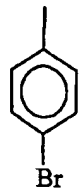


and L is H.

Still further the invention provides a compound wherein R_1 is CH_2CO_2K and R_2 is

and L is Br.

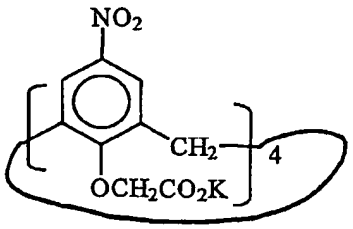
The invention also provides a compound wherein R_1 is CH_2CONH_2 and R_2 is



and L is Br.

The invention provides a method for the synthesis of a compound of Formula I above as outlined in Examples 1, 2, 5 and 6.

The invention further provides a compound of formula II for use in the preparation of a medicament for the treatment of viral infection, particularly HIV-1 infection.



Formula II

The compounds of formula I or II of the invention may be used in the preparation of a medicament for the treatment of viral infection, particularly HIV-1 infection.

The invention further provides a pharmaceutical composition comprising a pharmaceutically effective amount of a compound of formula I or II. The compounds of the present invention may be used in combination with pharmaceutically acceptable diluents or carriers to form pharmaceutical compositions for the treatment of viral infections, particularly HIV-1 infection.

Preferably, the invention provides a pharmaceutical composition comprising AZT and one or more of the compounds of formulae I and II.

The pharmaceutical composition according to the invention may comprise a compound of the invention together with a pharmaceutically effective carrier or excipient, and may be formulated as an injectable solution, a tablet, capsule, suppository or as a cream, gel or ointment for topical application.

The medicament may be used in the prevention of HIV-1 by using it in conjunction with a condom for example. The compounds of the invention have an improved selectivity index, over commercial topical spermicides and disinfectants, against HIV-1.

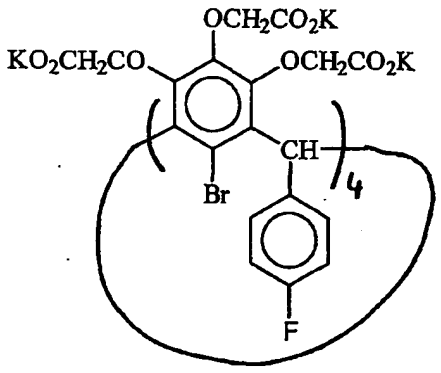
The invention provides a method of treatment of HIV infection comprising administering to a patient a pharmaceutically effective amount of at least one compound of formula I or II.

The invention also provides a method of treatment of HIV further comprising administering to a patient a pharmaceutically effective amount of at least one compound of formula I or II together with a pharmaceutically effective amount of AZT.

The invention will now be described in greater detail with reference to the following Examples.

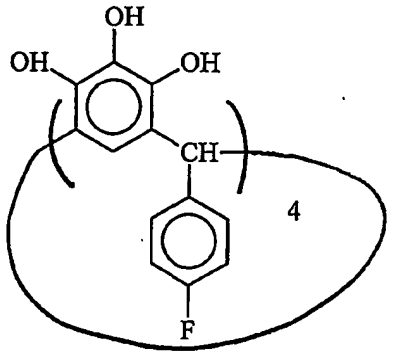
Detailed Description of the Invention

Example 1 AC-1



AC-1

Step (i) Preparation of:

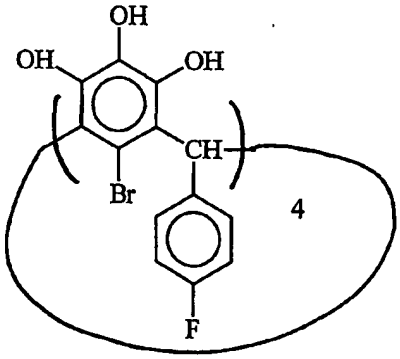


233g (1.88mole) of P-fluorobenzaldehyde was reacted with stirring with 236.5g (1.88 mole) pyrogallol in I.Il absolute ethanol and 275 mls 37% aqueous hydrochloric acid under reflux for 5 hours. After cooling the reaction mixture was filtered through scintered glass and the grey-brown solid collected was washed once with 4:1 absolute ethanol:water then filtered again and left to dry overnight in the oven at 60°C to give 286.6g pyrogallol P-F-phenyl tetramer product in 65% yield.

Elemental analysis calculated for $C_{52}H_{36}O_{12}F_4$:

C = 67.24, H =3.91% Found C=66.84, H=4.00%

Step (ii) Preparation of:



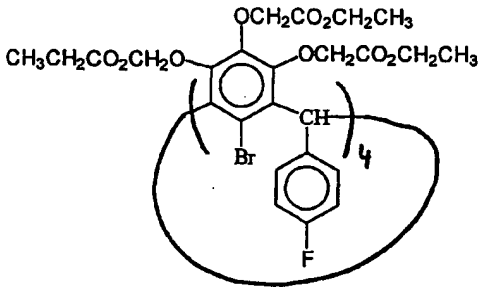
143.3g (0.15 mole) pyrogallol P-F-phenyl tetramer was added to 2l glacial acetic acid to which was added dropwise with stirring during 50 minutes 106.0g (0.66moles) elemental bromine under nitrogen at room temperature.

After addition the reaction mixture was stirred for 48 hours at room temperature under nitrogen, following which it was poured into a large volume of water in a well-ventilated area to precipitate the product.

The grey-brown solid was filtered through a grade 4 scintered glass funnel for filtering fine small particle solids and then washed once with water refiltered and dried in the dark for several days until perfectly dry at room temperature, occasionally manually breaking up lumps. The solid turns pink in the light. It is important that the product is perfectly dry at this point. Yield of bromo-pyrogallol P-F-phenyl tetramer is 132.0g (69%).

Elemental analysis calculated for $C_{52}H_{32}O_{12}F_4Br_4$: C=50.19%, H=2.59%
Found C=49.63% H=2.54%

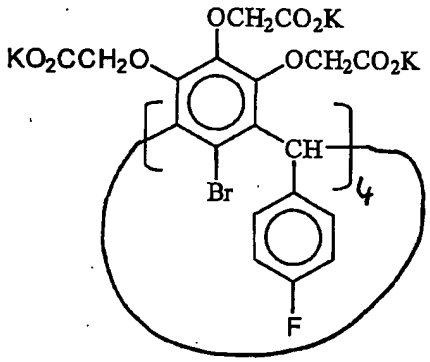
Step (iii) Preparation of:



160.0g (0.13mole) bromo-pyrogallol P-F-phenyl tetramer was reacted with 324g (2.3 mole) anhydrous potassium carbonate and 283g (1.69 mole) ethyl bromacetate (care lachrymator) with stirring in 5l dry HPLC acetone refluxed for 3 days with drying tube attached. After cooling to room temperature all volatiles were removed under reduced pressure employing a rotary evaporator (1l of reaction mixture at a time) then residue was treated with 230mls 37% aqueous hydrochloric acid mixed with 1l water. The slightly sticky red-brown solid was then washed with water and transferred to a glass dish in a 60°C oven. After a day or two at 60°C the product partially melts and water separates out which can be poured off to accelerate drying. After several more days the product hardens as it dries. This normally takes several more days. It is important that the solid is dry. When the solid is dry it is pulverised with a mortar and pestle to give 247g (80% yield) of the dodecaethylacetate of p-bromopyrogallol P-F-phenyl tetramer.

Elemental Analysis Calculated for $C_{100}H_{104}O_{36}F_4Br_4$: C=52.73%, H=4.60%, Found C=52.36%, H=4.23%.

Step (iv) Preparation of:

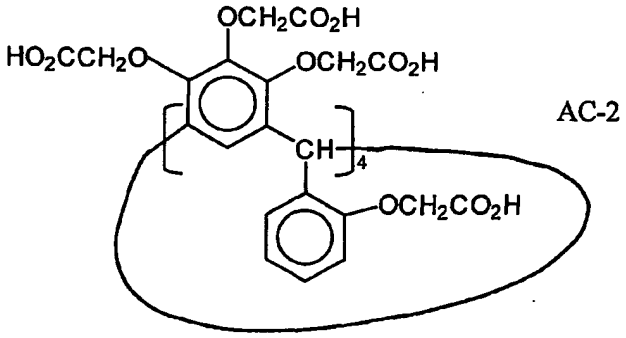


247g of the dodecaethylacetate of p-bromopyrogallol P-F-phenyl tetramer is stirred for 2 hours under reflux with drying tube attached with 200g KOH in 2l absolute ethanol then filtered hot through scintered glass and the red-brown solid immediately placed in a 60°C oven for 4 hours to give 260g product 100% yield of dodecapotassium acetate of p-bromopyrogallol P-F-phenyl tetramer as a red brown solid. AC-1

This product is somewhat hygroscopic and filtering hot and immediate transfer to 60°C oven renders a product much less prone to becoming sticky in the air than filtering at room temperature and leaving in the open air to dry at room temperature.

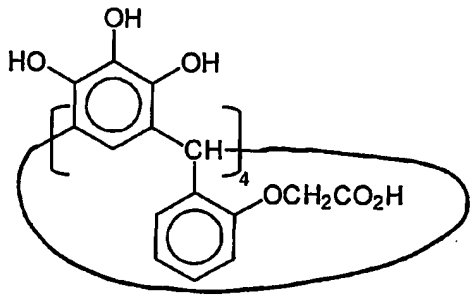
Elemental Analysis Calculated for $C_{76}H_{44}O_{36}F_4Br_4K_{12}$:
C=38.06% H =1.85%. Found C=38.07, H=2.30%

Example 2 AC-2



AC-2

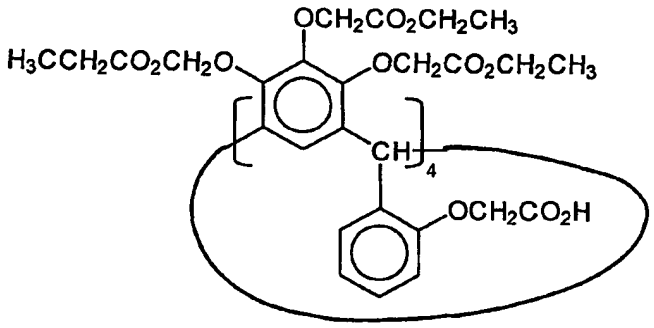
Step (i): Preparation of :



6.31g (0.05mole) pyrogallol and 9.01g (0.05mole) 2-formylphenoxy acetic acid were reacted together with stirring in 40mls absolute ethanol and 10mls 37% aqueous hydrochloric acid under reflux for 4 hours. After cooling the reaction mixture was filtered through scintered glass and the brown-grey solid was then washed with absolute ethanol:water 4:1 then filtered and dried overnight at 60°C in an oven to give 10.4g (72% yield) of pyrogallol 2-phenoxyacetic acid tetramer.

Elemental analysis calculated for $C_{60}H_{48}O_{24}$:
C=62.50, H=4.20%, Found C=62.01, H=4.10%

Step (ii): Preparation of:

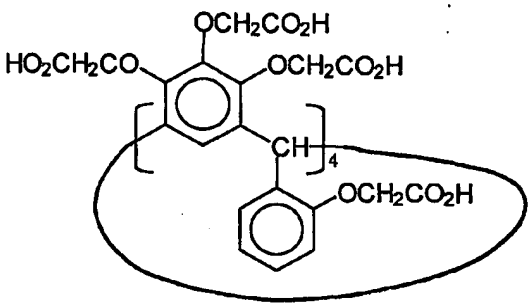


To 10.4g pyrogallol 2-phenoxyacetic acid tetramer (0.009 mole) was added 30.3g (0.220 mole) anhydrous potassium carbonate and 19.9g (0.120 mole) ethyl bromoacetate (care lachrymator) in 390mls dry HPLC acetone and the entire was refluxed for 3 days with

drying tube attached. After this time all volatiles were removed and to the residue was added 22mls 37% aqueous hydrochloric acid mixed with 100mls water.

After breaking up the solid and stirring well the reaction mixture was filtered through scintered glass and then washed with water filtered and dried in 60°C oven for 5 days to give dodecaethyl acetate of pyrogallol 2-phenoxyacetic acid tetramer 14.3g (73% yield). Elemental Analysis calculated for $C_{108}H_{120}O_{48}$ C=59.33, H=5.53%, Found C=59.10, H=5.21%

Step (iii): Preparation of:



14.3g (0.06 mole) dodecaethyl acetate of pyrogallol 2-phenoxyacetic acid tetramer was added to 14.3g KOH in 140ml absolute ethanol and the entire was refluxed with drying tube attached for 3½ hours. After filtering hot through scintered glass the solid* was immediately added to 50ml glacial acetic acid in 175mls absolute ethanol with stirring and filtered through scintered glass then immediately placed in 60°C oven for several hours to give 12.0g (99% yield) dodeca-acetic acid of pyrogallol-2-phenoxyacetic acid tetramer AC-2 which is very hygroscopic (picks up moisture from air) and should be stored in sealed container as a purple brown solid quickly becoming sticky in the air. The product contains some potassium acetate from the neutralisation of the hexadeca potassium acetate pyrogallol tetramer with acetic acid

Elemental analysis calculated for $C_{84}H_{72}O_{48}.16CH_3CO_2K$:

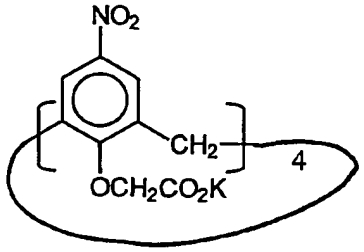
C=40.74, H=3.54% Found C=40.30, H=3.81%

* A small sample was added to 20% aqueous HCl which was then placed in a fridge. After one week colourless crystals of pure product were obtained.

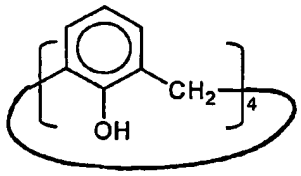
Elemental Analysis calculated for $C_{84}H_{72}O_{48}.6H_20$:

C=51.53, H=4.32% Found C=51.07, H=4.32%

Example 3 - AC-3 Route A



Step (i): Preparation of:

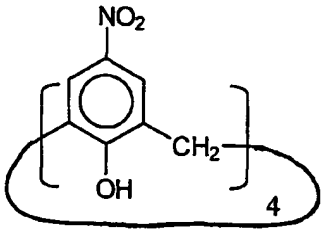


A slurry of 13.3g (0.020 mole) p-t-butylcalix-4-arene, 9.02g (0.096 mole) phenol* and 14g (0.015 mole) anhydrous $AlCl_3$ was stirred in 125mls toluene at room temperature for 1 hour under nitrogen with drying tube attached. The mixture was then poured into 250mls 0.2N HCl, the organic phase was separated and toluene was removed under reduced pressure on a rotary evaporator. Upon the addition of methanol a precipitate formed which was removed by filtration to give 7.54g 89% yield calix-4-arene as an off-white solid which was dried well in an oven at 60°C for several hours. It is important that the product is dry (dry overnight 60°C).

*May be left out but the reaction time is then 1 day at room temperature. Ref: C.D.Gutsche and L.Lin Tetrahedron 42 6 1986 p1633

Elemental analysis calculated for $C_{28}H_{24}O_4$: C=79.22, H=5.70% Found C=79.10, H=5.60%

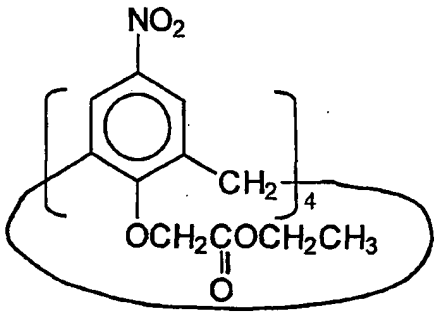
Step (ii): Preparation of:



5.0g (0.012mole) calix-4-arene was stirred in 50mls concentrated sulphuric acid at 95°-100°C for 10 hours. After cooling the reaction mixture was diluted with 90mls cold water (external cooling required - care) and then entire was cooled to 0°C and then treated with 5.8g 61% HNO_3 for 10 hours at 0°C with stirring. The entire was then diluted with water ca 500ml and a fine yellow solid product precipitated which was filtered off then washed once with water then dried in 60°C for several hours.
It is important that the product is dry. Yield =7.1g (99% yield) as a pale yellow green solid p-nitrocalix-4-arene. (Dry in oven at 60°C overnight).

Procedure adapted from S.Shinkai, T.Tsubaki, T. Sone and O.Manabe, Tetrahedron Letters 26(28) p3343 1985
Elemental analysis calculated for $C_{28}H_{20}N_4O_{12}$
C=55.63, H=3.34% Found C=55.13, H=3.66%

Step (iii) Preparation of:



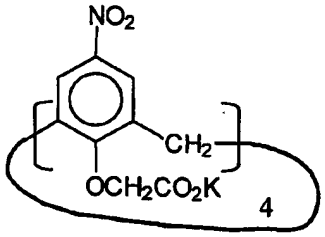
11.2g (0.019 mole) p-nitrocalix-4-arene was added to 15.5g (0.11 mole) anhydrous granular potassium carbonate, 13.0g (0.078 mole) ethyl bromoacetate (care lachrymator) and 200mls dry HPLC acetone and the entire was refluxed with drying-tube attached for

three days. After cooling all volatiles were removed under reduced pressure on a rotary evaporator and to residue was added 110mls 10% aqueous HCl (hydrochloric acid). The solid was well broken up and stirred well then filtered off and the solid was washed with water then dried for several hours in an oven at 60°C.

It is important to dry the product thoroughly again. Yield 15.7g (87%). The tetraethyl acetate of p-nitrocalix-4-arene (dry 60°C oven overnight).

Elemental analysis calculated for $C_{44}H_{44}N_4O_{20}.5H_2O$. C=51.26, H=4.50% Found C=51.25, H=3.99%

Step (iv) Preparation of:



17.2g (0.018 mole) tetraethylacetate of p-nitrocalix-4-arene was refluxed with 17g KOH in 111g absolute ethanol for two hours with drying tube attached. After this time the reaction mixture was filtered through scintered glass while still hot to give 17.7g (99%) of potassium acetate of p-nitrocalix-4-arene as a red-brown solid AC-3 which was dried in an oven at 60°C for a few hours.

Elemental analysis calculated for $C_{36}H_{24}N_4O_{20}K_4$: C=43.72, H=2.45% Found C=43.37, H=2.90%

Example 4 AC-3 - Route B

To 2.99g (0.003 mole) t-butylcalix-4-arene tetraethyl acetate in 30 mls dichloromethane and 30mls glacial acetic acid cooled to and kept at 0°C was added with stirring 10ml (0.240 mole) 100% nitric acid HNO_3. The reaction mixture was stirred then for 40 minutes at 0°C and subsequently allowed to warm to room temperature and stirred until the black-purple colour had discharged. It was then poured into 200mls water. The water layer was extracted with 2 x 50mls dichloromethane. The combined organic layers were

washed with water 2 x 30mls then dried with dried magnesium sulphate then volatiles removed to give product which was then recrystallised from dichloromethane/petroleum ether 40°C-60°C to give 1.06g (37% yield*) p-nitrocalix-4-arene tetraethyl acetate which was converted into AC-3 using the procedure as outlined in method A, step (iv).

5

*Ref: W. Verboom, A. Durie, R.J.M. Egberink, Z. Asfari and D.N. Reinhoudt, J. Org. Chem. 57 1992 p1313.

Example 5

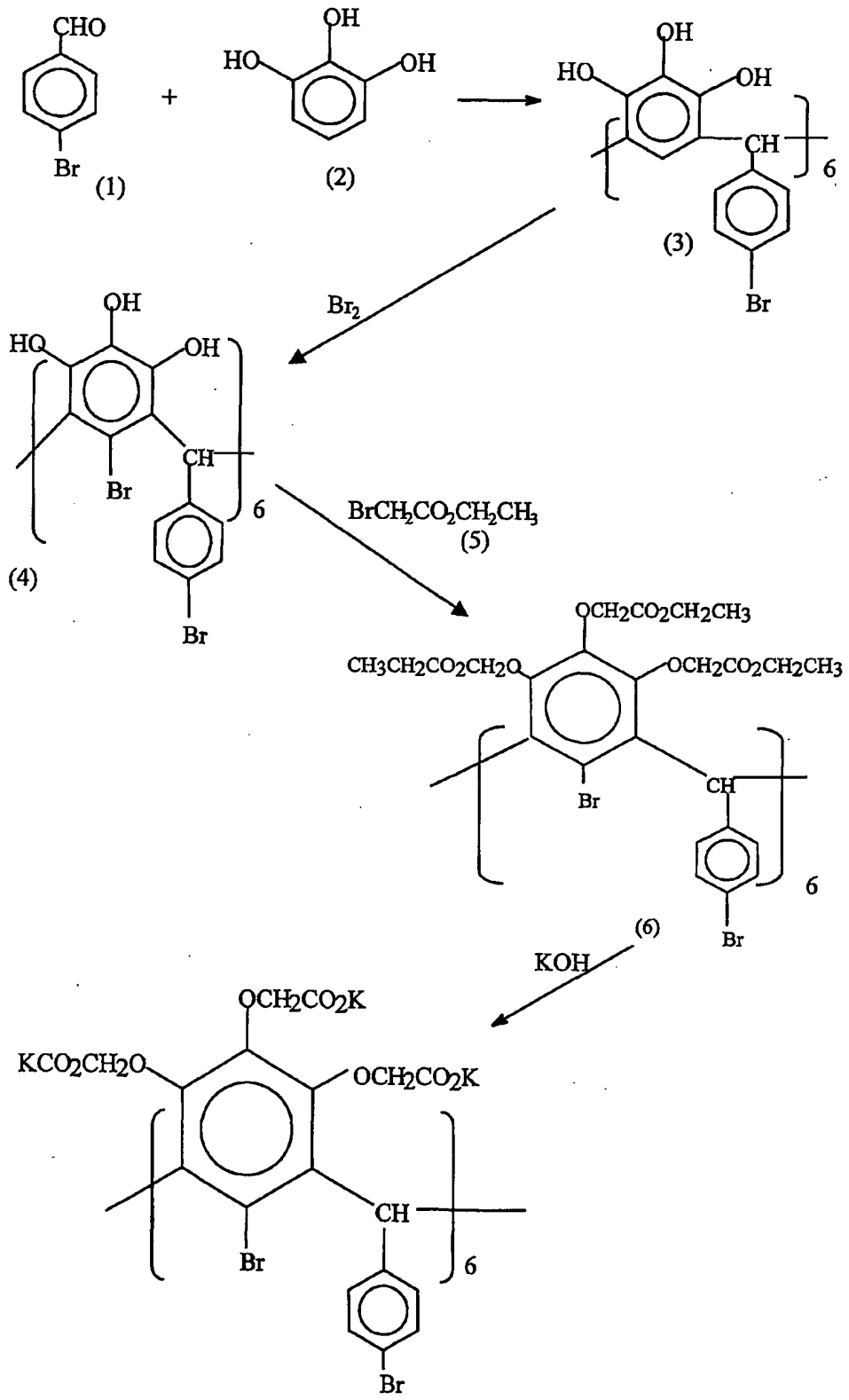
240g (1.30 mole) of P-bromobenzaldehyde (1) was reacted with stirring with 163.5g (1.30 mole) pyrogallol (2) in 1.5 litres absolute ethanol and 215mls 37% aqueous hydrochloric acid under reflux for 4 hours. After cooling the reaction mixture was filtered through scintered glass and the brown solid collected and washed with 4:1 ethanol:water before drying at 70°C to give 266g pyrogallol P-Br-phenyl hexamer product (3) in 70% yield.

100g (0.06 mole) of P-Br-phenyl hexamer (3) was added to one litre glacial acetic acid to which was added drop wise with stirring during 30 minutes 64.2g (0.4 moles) elemental bromine under nitrogen at room temperature. After addition the reaction mixture as stirred for 24 hrs at room temperature under nitrogen, following which it was poured into a large volume of water in a well-ventilated area to precipitate the product. The brown solid was filtered through a grade 4 scintered glass funnel for filtering fine small particle solids and then washed once with water refiltered and dried in the dark at room temperature. Yield of bromo-pyrogallol P-Br-phenyl hexamer (4) is 89g (60%).

80g (0.04 mole) Bromo-pyrogallol P-Br-phenyl hexamer (4) was reacted with 113g (0.8 mole) anhydrous potassium carbonate and 130.2g (0.78 mole) ethylbromoacetate (5) with stirring in 2 litres of HPLC grade acetone and refluxed for 24 hours. After cooling to room temperature all volatiles were removed under reduced pressure employing a rotary evaporator and the residue treated with 150mls 37% aqueous hydrochloric acid mixed with 800mls of water. The resultant brown product was dried at 60°C to give 128.5g (85% yield) of the ethylacetate of p-bromopyrogallol P-Br-phenyl hexamer (6).

128g (0.034 mole) of the ethylacetate of p-bromopyrogallol P-Br phenyl hexamer (6) is stirred for two hours under reflux with 48g KOH in one litre absolute ethanol and filtered hot through scintered glass. The resulting brown solid of the potassium acetate salt of p-bromopyrogallol P-Br-phenyl tetramer (7) (95% yield - 144g) is dried at 60°C.

Example 5


CHO
Br
(1)
+
OH
HO
OH
(2)
OH
HO
OH
CH
6
(3)
Br
Br_2
OH
HO
OH
CH
Br
6
(4)
Br
$BrCH_2CO_2CH_2CH_3$
(5)
$OCH_2CO_2CH_2CH_3$
$CH_3CH_2CO_2CH_2O$
$OCH_2CO_2CH_2CH_3$
Br
CH
6
Br
(6)
KOH
OCH_2CO_2K
KCO_2CH_2O
OCH_2CO_2K
CH
Br
6
Br

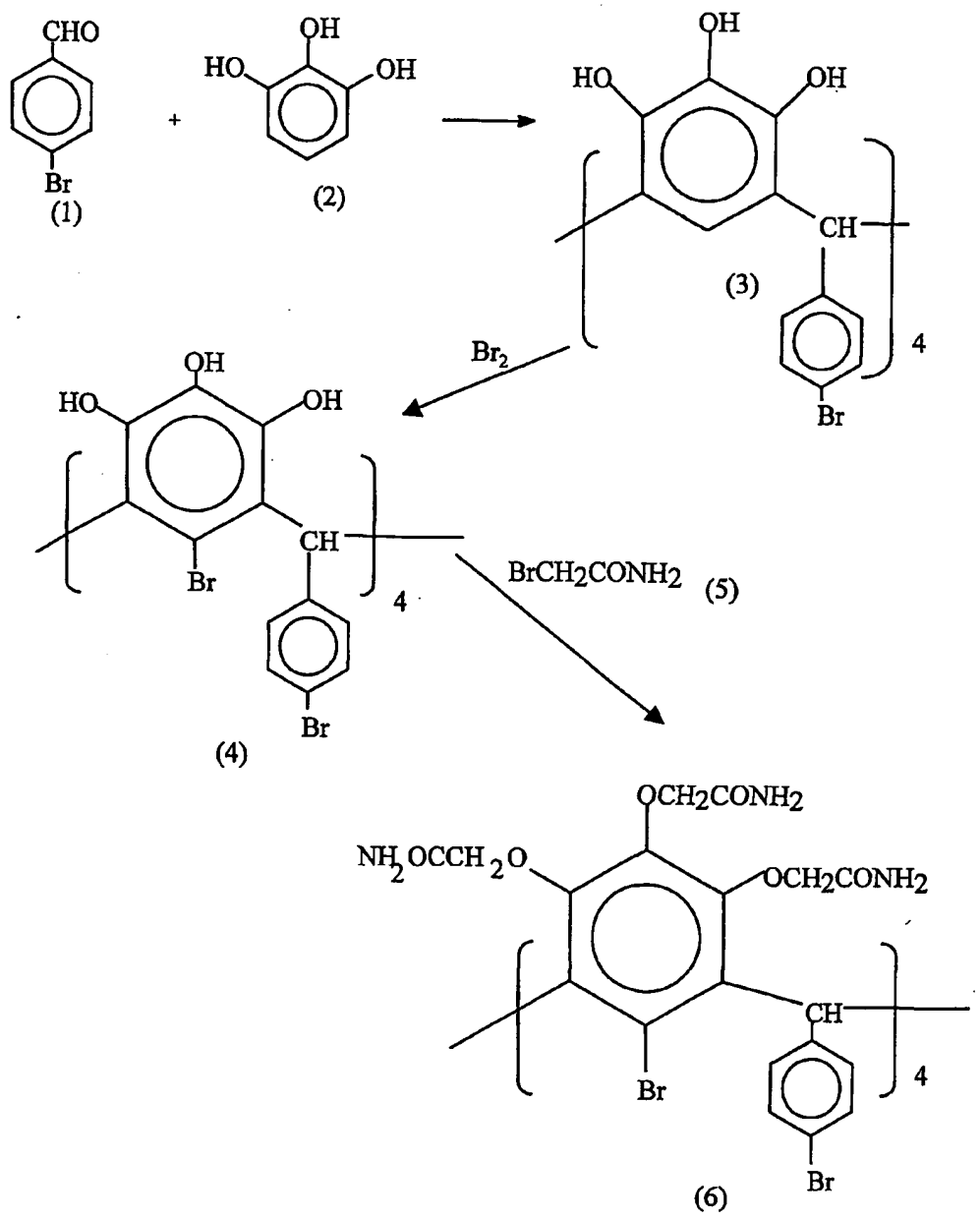
Example 6

250g (1.35 mole) of P-bromobenzaldehyde (1) was reacted with stirring with 170.1g (1.35 mole) pyrogallol (2) in 1.7 litres absolute ethanol and 225mls 37% aqueous hydrochloric acid under reflux for 3 hours. After cooling the reaction mixture was filtered through scintered glass and the brown solid collected and washed with 4:1 ethanol:water before drying at 70°C to give 297g pyrogallol P-Br-phenyl tetramer product (3) in 75% yield.

100g (0.085 mole) of P-Br-phenyl tetramer (3) was added to 800mls of glacial acetic acid to which was added drop wise with stirring during 30 minutes 29.5g (0.0187 moles) elemental bromine under nitrogen at room temperature. After addition the reaction mixture was stirred for 36 hrs at room temperature under nitrogen, following which it was poured into a large volume of water to precipitate the product. The brown solid was filtered through a grade 4 scintered glass funnel for filtering fine small particle solids and then washed once with water, refiltered and dried in the dark for several days until dry. Yield of bromo-pyrogallol P-Br-phenyl tetramer (4) to 89g (70%).

80g (0.054 mole) Bromo-pyrogallol P-Br-phenyl tetramer (4) was reacted with 113g (0.713 mole) anhydrous potassium carbonate and 96.6g (0.700 mole) 2-bromoacetamide (5) with stirring in 1.5 litres of HPLC grade acetone and refluxed for 12 hours. After cooling to room temperature all volatiles were removed under reduced pressure employing a rotary evaporator and the residue treated with 100mls 37% aqueous hydrochloric acid mixed with 400mls of water. The resultant brown product was dried at 60°C to give 93.8g (80% yield) of the dodecaacetamide of p-bromopyrogallol P-Br-phenyl tetramer (6).

Example 6


CHO
Br
(1)
+
OH
HO
OH
(2)
OH
HO
OH
CH
(3)
4
Br
Br_2
OH
HO
OH
CH
Br
4
Br
(4)
$BrCH_2CONH_2$ (5)
OCH_2CONH_2
NH_2OCCH_2O
OCH_2CONH_2
CH
Br
4
Br
(6)

5

Clinical Results

Anti-HIV Activity

Determination of EC_{50} and TC_{50}

Antiviral Assays

The anti-HIV and anti-SIV (Simian immunodeficiency virus) activities and toxicities of compounds were assessed in C8166 cells infected with HIV-1_{111B}, HIV-2_{ROD}, SIV_{MAC}. The cells are cultured in RPMI 1640 with 10% calf serum.

Aliquots of 4 x 10^4 cells per microtiter plate well were mixed with 5 fold dilutions of compounds prior to addition of 10 $CCID_{50}$ units of virus and incubated for 5-6 days. Formation of syncytia was examined from 2 days post-infection. Gp120 antigen produced at 5-6 days was measured by ELISA, using the lectin GNA (from Galanthus nivalis) to capture the glycoprotein and human anti-HIV serum for detection (9). Cell viability of infected, and uninfected control cells was measured by the MTT-Formazan method (10).

Briefly, 10 µl of a solution of 3-(4,5-dimethylthiazol-2-YL)-2,5 diphenyl-tetrazolium bromide (MTT, 7.5 mg/ml in PBS) was added to each well containing 100 µl of infected or uninfected cells. After incubating at 37°C for 1 hour, the blue formazan crystals produced are solubilized in 150 µl of 10% V/V triton X-100 in acidified isopropanol (2ml concentrated HCl per 500ml solvent) and absorbance read at 540nm.

gp 120 Antigen Assay

A microtiter antigen capture ELISA was developed using lectin (GNA) from Galanthus nivalis (Vector Laboratories, Peterborough, U.K.) and human antibodies (10). The plates were coated with lectin (0.5 ug), and after blocking with 10% calf serum, dilutions of virus supernatant in 0.25% detergent solution (Empigen, Albright and Wilson Ltd., Whitehaven, U.K.) were added to the wells and incubated at 4°C for 12-16 hours. Bound antigen was captured using human anti-HIV antibodies, and finally detected with anti-human Ig antibodies conjugated to horseradish peroxidase.

EC_{50} represents the concentration which reduces the Ag gp120 by 50% in infected cell cultures.

TC_{50} represents the concentration of drug which reduces cell growth by 50%.

Table 1

Compound	EC_{50} µm	TC_{50} µm	TC_{50}/EC_{50} µm
AZT	0.016	>1000	>62,500
AC-1	0.1	400	4,000
AC-2	1-2	100	50-100
AC-3	1	>100	>100

A comparison of the Therapeutic Index of the compounds of the invention with that of AZT would suggest that these compounds especially AC-2 and AC-3 are not worth testing in vivo and actually teaches away from the invention. In general researchers like to see a Therapeutic Index of thousands before progressing a drug to clinical trial. However, the present inventor has surprisingly found that these compounds are significantly more effective in vivo than AZT.

In vitro studies anti-HIV-1 activity

An additive effect with AZT was demonstrated for AC-1, AC-2 and AC-3. AZT inhibits reverse transcriptase enzyme. The drugs of the present invention inhibit fusion and integrase enzyme. Also there was an additive effect for the three combinations of two of the drugs of the present invention *in vitro*. Table 2 illustrates the additive effect.

All three drugs are believed to act against HIV-1 in the "fusion" stage of its life cycle.

Table 2

Combination of Compounds	$EC_{50}\mu m$
AC-1 + AC-2	0.05 + 0.7
AC-1 + AC-3	0.04 + 0.5
AC-2 + AC-3	0.7 + 0.5
AC-1 + AZT	0.03 + 0.005
AC-2 + AZT	0.5 + 0.007
AC-3 + AZT	0.4 + 0.006

Table 3

Integrase activity (Ref N.Neamati, S.Sunder and Y. Pommier, Drug Discovery Today 2 (11) 1997 p487)

Integrase Activity	**$EC_{50}(\mu m)$ /3'Processing**
AC-3	6.2
AC-1	14.3
AC-2	15.9

Each drug was administered orally to patients at a level of 500mg per day (three doses of 167mg each 8 hours). It was found that each drug reduced viral loads in the patients blood by an average of 2 log units i.e. 100 times. The viral load was measured using a Roche Amplicor. A Roche Amplicor is a commercial apparatus for measuring viral load in patients blood measured in copies per ml of blood which means the number of free HIV-1 viral particles per millilitre of patient's blood. This quantitative method is based on PCR (Polymerase Chain Reaction) and is FDA-approved.

All patients also registered a significant increase in CD4 expressing cells which is an indication of improved immune status. A Becton Dickinson Flowcytometer was used to measure the level of CD4 cells. This apparatus is commercially available and is used to measure levels of cells in the blood including CD4 T cellls. A blood sample of known volume is taken from the patient and the white blood cells are separated off. These are then suspended in a liquid to which are added flourescent-labelled monoclonal antibodies specific for the type of blood cell whose level is needed to be measured e.g.

CD4 T cells, to which they become attached by binding to the specific protein present in the cell such as CD4. The result is a suspension of flourescent-labelled-CD4 cells in this case which are passed through an exceedingly fine nozzle in the Becton Dickinson Flowcytometer which is electrically charged and which allows only one flourescent - labelled cell to pass at a time which are then directed to a flourescent light detector-analyser attached to a computer. The total light change detected arising from the total number of cells present reveals the number of flourescent-labelled CD4 T cells and hence the number of CD4 T cells present per ml of blood.

The patients treated using the drugs of the present invention were all with an advanced stage of the disease. In particular, one female patient had, prior to treatment, 500,000 viral copies (free viral particles of HIV-1) per ml of blood. After treatment with AC-1 her viral loads were reduced to 1% of the original) and were maintained for at least a year and were found to be fairly constant during this time period. A man with 750,000 viral copies per ml of blood was found 3 months later after treatment with AC-1 to have 50,000 viral copies with great improvement in clinical condition. Patients being treated with the drugs of the present invention did not experience any side effects. Furthermore, their clinical condition dramatically improved in all cases.

In addition, there was a significantly reduced incidence of opportunistic infections and improvement in physical fitness (Karnofsky Score) in all patients. The "Karnofsky score" is a measure of physical fitness on a scale of 0 to 100. A score of 0 refers to a person being dead and a score of 100 is a 'normally' fully fit individual with all his faculties in order able to work full-time and carry out activities associated with a 'normal' lifestyle. A disabled individual unable to walk and confined to a wheel-chair with normal sight and hearing would merit a score of about 70. An individual on a "ventilator" i.e. with his breathing needing assistance and in "intensive care" would be given a score of about 30.

The drugs according to the present invention inhibit fusion and integrase enzyme.

Table 4 *In vitro* efficiency tests

Compound	Conc Um	Syncytia (+/-)	Antigen p24 % Control	Estimated Cell Growth % of Control		EC_{50}	TC_{50}	SI TC/EC
				Infected	Uninfected			
1151C	400	-		73	69	0.25	500	2000
AC-1	80	-		105	100			
(~60%	16	-		92				
purity)	3.2	-		92				
	0.64	-/+	16.8	72				
	0.128	-/+	63	30				
	0.0256	+		28				
04-002	400	T50		41	42	0.015	350	23333
AC-1	80	-		93	94			
(~80%	16	-		99				
purity)	3.2	-		104				
	0.64	-		101				
	0.128	-	14	100				
	0.0256	-/+	30	46.5				
04-003	400	T50		44	45	0.012	350	29166
AC-1	80	-		100	100			
(~85%	16	-		96				
purity)	3.2	-		100				
	0.64	-		100				
	0.128	-	0	100				
	0.0256	-/+	25	55				
04-004	400	T40		41	41	0.32	350	1100
Product	80	-		100	100			
Inter-	16	-		100				
mediate 1	3.2	-		94				
	0.64	-/+	27	70				
	0.128	+/-	99	34				
	0.0256	+		29				
AC	200	T		16	16	8	30	3.75
Product	40	T25		29	31			
Inter-	8	-/+	54	58	98			
mediate 2	1.6	+	99	27	100			
	0.32	+		27				
	0.064	+		24				
AZT	2	-		100	100	0.016	>1000	>62,500
	0.016	+/-	51	49				
Control	0	+	100	24	100			

EC_{50} represents the concentration which reduces the viral antigen p24 50% in infected cell cultures.

TC_{50} represents the concentration of drug which reduces cell growth by 50%.

Column 3 = Syncytia is examined microscopically where infected cells fuse and produce distinct giant cells.

Column 4 = Drug treated infected cell supernatant is removed and virus related antigen p24 is measured by ELISA. Untreated infected control is taken as 100%.

Column 5 = Cell growth (infected and uninfected) is measured by MTT-formazan method. Higher number of cells in infected cultures indicates protective effect of compounds and uninfected cell controls show drug toxicity.

Compounds 04-002, 04-003, 04-004 and 1151C were dissolved in water at 20 mM diluted 1/10 in growth medium for testing.

Compound AC- was dissolved in DMSO at 50 mM and diluted 1/100 in medium for testing, DMSO is not toxic to cells at 1% concentration.

Compounds 04-002 and 003 are at least 10 times better than 1151C and 04-004.

The drugs demonstrated an improved performance over that known for AZT. AZT is known to reduce viral loads in patients by 50-90%, that is up to 1 log unit (ten times). However continued oral administration of AZT leads to quickly leads to the development of viral drug resistance.

The drugs of the present invention bring about a reduction in viral load similar to that exhibited by daily oral monotherapy with Merck Sharp and Dohme's protease inhibitor 'indinavir' (three doses of 800mg each 8hours i.e. 2400 mg per day - much higher dose needed!), or Hoffmann-La Roche's protease inhibitor 'saquinavir' (three doses of 600 mg each 8 hours i.e. 1800 mg per day) or 'ritonavir' (three doses of 600 mg each 8 hours i.e.1800 mg per day). However, the drugs of the present invention do not exhibit the 6 month development of viral drug resistance which accompanies the use of these protease inhibitors when used alone i.e. in monotherapy. Therefore, protease inhibitors have to be used in combination with others to prevent development of viral drug resistance.

During a year's treatment with the drugs of the present invention viral loads were reduced by up to 99%. Unlike the protease inhibitors no side effects were observed.

Additionally, no viral drug resistance was observed during daily oral administration of each drug over a period of a year (500mg a day).

This could well be due to the fact that the compounds of the present invention appear to act at two different stages in the life cycle of the HIV-1 virus. The drugs of the present invention appear to act on the early fusion stage and later integrase stage of the life cycle.

An important advantage of the compounds of the present invention is their relatively low cost and ease of synthesis. They are especially suitable for use in the developing world.

The drugs of the present invention offer advantages over those of the prior art in that they are lower in cost and a lower dose of drug is required. The high water solubility may give advantages over protease enzyme inhibitors in that they have superior absorption by the body and a higher degree of bioavailability.

In addition use of only one drug avoids the complications of a strict regime of the order of taking a combination of drugs which must be strictly adhered to by the patient. All patients treated with AC-1, AC-2 and AC-3 experienced increased CD4 T cell levels after treatment.

MARKETPLACE COMPARISON						
DRUG (NEW)	**TYPE**	**REDUCTION IN VIRAL LOAD**	**TOTAL AMOUNT DRUG/DAY** in (x) doses	**SIDE EFFECTS**	**VIRAL DRUG RESISTANCE (MTHS)**	**COST/ PATIENT/YEAR (PUNTS) COMPANY**
AC-1	fusion, integrase enzyme inhibitor	99%	500mg (3)	none observed during one year	none observed during one year	Aids Care Pharma Ltd.
AC-2	fusion, integrase enzyme inhibitor	99%	500mg(3)	none observed during one year	none observed during one year	Aids Care Pharma Ltd.
AC-3	fusion, integrase enzyme inhibitor	99%	500mg(3)	none observed during one year	none observed during one year	Aids Care Pharma Ltd.

Viricidal Anti-HIV Activity

AC-1, AC-2 and AC-3 were tested as vaginal viricides against HIV-1. An effective vaginal viricide would need to have high anti-HIV-1 activity, low toxicity, stability at relatively high ambient temperatures and at pH 4-7, absence of odour and taste and cheapness (Private Communication from H.Pask, AIDS Secretariat Medical Research Council, London, England). The drugs of the present invention were tested following the method of T.J. O'Connor, D. Kinchington, H.O. Kangro and D.J. Jeffries, 'The Activity of Candidate Viricidal Agents low pH and Genital Secretions against HIV-1 'in vitro' in International Journal of STD and AIDS 6 1995 p267.

Table 5

Compound	$IC_{50}\mu m$	$CC_{50}\mu m$	$CC_{50}\mu m/ IC_{50}\mu m$ Selectivity Index
AC-1	1.1	>50	>43.4
AC-2	5.5	>50	>9
AC-3	5.5	>50	>9
nonoxynol-9	8	11	1.6
octoxynol	0.11μg/ml	0.31μg/ml	2.8
benzalkonium chloride	0.12μg/ml	0.60μg/ml	5.0
chlorhexidine	0.25	1.3	5.2

IC_{50} is the concentration of compound giving 50% inhibition of HIV-1 p24 antigen

CC_{50} is the concentration of compound giving 50% of cell growth (cytotoxicity)

nonoxynol-9 is a commercial spermicide of Cilag Ltd.

octoxynol is a commercial spermicide from ICI surfactants

benzalkonium chloride is a commercial spermicide from Fluka

chlorhexidine is a commercial disinfectant from Sigma

The selectivity index of AC-1, AC-2, AC-3 is >43.4, >9, >9 which is superior to the three commercial spermicides: nonoxynol-9, octoxynol and benzalkonium chloride 1.6, 2.8 and 5.0 and that of the disinfectant chlorhexidine 5.2.

The words "comprises/comprising" and the words "having/including" when used herein with reference to the present invention are used to specify the presence of stated features, integers, steps or components but does not preclude the presence or addition of one or more other features, integers, steps, components or groups thereof.

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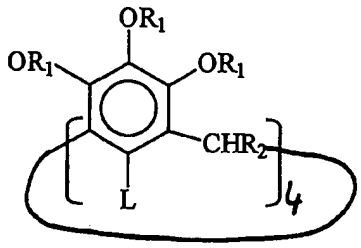
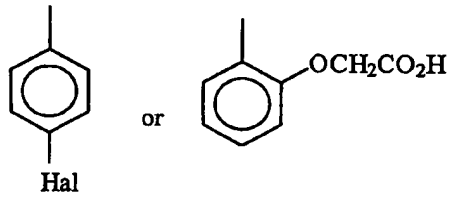
Claims

1. A compound of formula I



Formula I

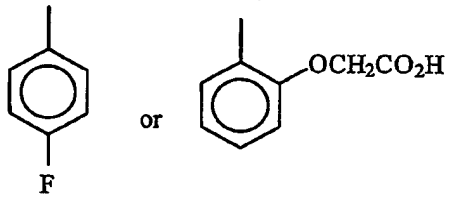
wherein R_1 is CH_2CO_2K, CH_2CO_2H or CH_2CONH_2 and R_2 is



where Hal is a halogen, preferably F or Br, and L is H or a halogen, preferably Br.

2. A compound of Formula I as claimed in claim 1 wherein R_1 is CH_2CO_2K or CH_2CO_2H,

R_2 is

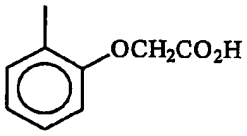


and L is H or a halogen, preferably Br.

3. A compound according to claim 1 wherein R_1 is CH_2CO_2K, R_2 is

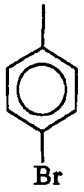
and L is Br.

4. A compound according to claim 1 wherein R_1 is CH_2CO_2H, R_2 is



and L is H.

5. A compound according to claim 1 wherein R_1 is CH_2CO_2K and R_2 is

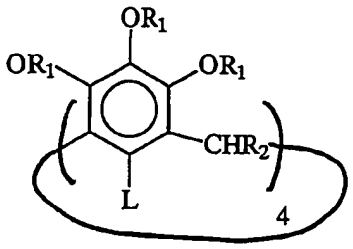


and L is Br.

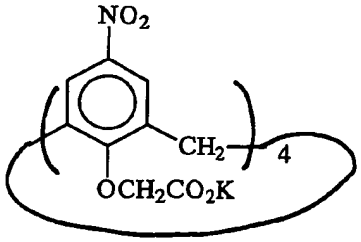
6. A compound according to claim 1 wherein R_1 is CH_2CONH_2 and R_2 is

and L is Br.

7. Use of a compound of formula I or II

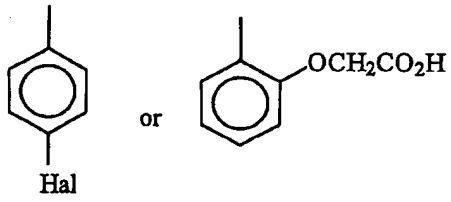


Formula I



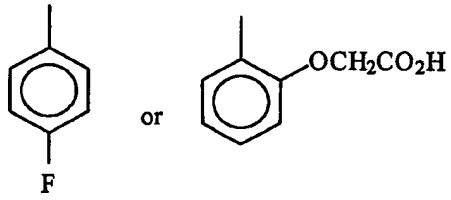
Formula II

wherein R_1 is CH_2CO_2K, CH_2CO_2H or CH_2CONH_2 and R_2 is



where Hal is a halogen, preferably F or Br, and L is H or a halogen, preferably Br, in the preparation of a medicament for the treatment of viral infection, particularly HIV-1 infection.

8. Use as claimed in claim 7 wherein R_1 is CH_2CO_2K or CH_2CO_2H, R_2 is



and L is H or a halogen, preferably Br.

9. A pharmaceutical composition comprising a pharmaceutically effective amount of a compound of formula I or II as defined herein together with a pharmaceutically acceptable carrier or diluent.

10. A pharmaceutical composition comprising AZT and one or more of the compounds of formulae I and II as defined herein together with a pharmaceutically acceptable carrier or diluent.

11. A method of treatment of HIV infection comprising administering to a patient a pharmaceutically effective amount of at least one compound of formula I or II.

12. A method as claimed in claim 11 further comprising administering a pharmaceutically effective amount of AZT.

13. A method for the synthesis of a compound according to claim 3 or 5 comprising the steps of

(i) condensing pyrogallol with aldehyde in equimolar quantities in refluxing 37% aqueous HCl/ethanol to form a halogenated oligomer, washing the precipitated oligomer with 4:1 ethanol:water and drying;

(ii) adding bromine dropwise to said oligomer in glacial acetic acid under nitrogen at room temperature

(iii) following addition of bromine, stirring reaction mixture at room temperature under nitrogen; pouring reaction mixture into water to precipitate the product; filtering the product to yield bromo-pyrogallol P-Hal-phenyl oligomer washing, refiltering and drying, preferably in the dark;

(iv) reacting said bromo-pyrogallol P-Hal-phenyl oligomer with anhydrous potassium carbonate and ethyl bromacetate in refluxing dry acetone, allowing to cool to room temperature removing all volatiles under reduced pressure;

(v) treating residue with 37% aqueous HCl mixed with water, drying, pulverising to form dodecaethylacetate of p-bromopyrogallol P-Hal- phenyl oligomer

(vi) stirring dodecaethylacetate of p-bromopyrogallol P-Hal- phenyl oligomer with KOH in refluxing ethanol to yield dodecapotassium acetate of p-bromopyrogallol P-Hal-phenyl oligomer.

14. A method according to claim 13 wherein said oligomer comprises 4 or 6 monomer units.

15. A method according to claim 13 or 14 wherein Hal is F or Br.

16. A method for the synthesis of a compound according to claim 6 comprising the steps of

(i) condensing pyrogallol with aldehyde in equimolar quantities in refluxing 37% aqueous HCl/ethanol to form a halogenated tetramer, washing the precipitated tetramer with 4:1 ethanol:water and drying;

(ii) adding bromine dropwise to said tetramer in glacial acetic acid under nitrogen at room temperature

(iii) following addition of bromine, stirring reaction mixture at room temperature under nitrogen; pouring reaction mixture into water to precipitate the product; filtering the product to yield bromo-pyrogallol P-Br-phenyl tetramer washing, refiltering and drying, preferably in the dark;

(iv) reacting said bromo-pyrogallol P-Br-phenyl tetramer with anhydrous potassium carbonate and 2-bromoacetamide in refluxing dry acetone, allowing to cool to room temperature removing all volatiles under reduced pressure;

(v) treating residue with 37% aqueous HCl mixed with water, drying, pulverising to form dodecaacetamide of p-bromopyrogallol P-Br- phenyl tetramer.

17. A method for the synthesis of a compound according to claim 4, comprising the steps of

(i) reacting equimolar quantities of pyrogallol and 2-formylphenoxyacetic acid in refluxing 37% HCl/ethanol 1/4 by volume , filtering and washing with absolute ethanol:water 4:1, filtering and drying to yield pyrogallol 2-phenoxyacetic acid tetramer;

(ii) reacting said tetramer with anhydrous potassium carbonate and ethyl bromoacetate in refluxing dry acetone; removing all volatiles and adding to the residue a minimum quantity of 37% aqueous HCl mixed with water, filtering and washing with water, filtered and dried to yield dodecaethyl acetate of pyrogallol 2-phenoxyacetic acid tetramer;

(iii) reacting dodecaethyl acetate of pyrogallol 2-phenoxyacetic acid tetramer with KOH in ethanol and refluxing;

(iv) filtering hot and adding immediately to glacial acetic acid in ethanol with stirring; filtering and immediately placing in an oven to yield dodeca-acetic acid of pyrogallol-2-phenoxyacetic acid tetramer.

INTERNATIONAL SEARCH REPORT

Int onal Application No
PCT/IE 01/00150

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C59/70 C07C205/37 C07C235/18 A61K31/194 A61K31/165
A61P31/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BEILSTEIN Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 19974 A (HARRIS STEPHEN J) 27 July 1995 (1995-07-27) cited in the application examples 40,211,212 examples 217,218 page 12, line 20 -page 15, line 5	1-17
A	US 5 312 837 A (HWANG KOU M ET AL) 17 May 1994 (1994-05-17) figure 8 column 3, line 26 -column 4, line 60	1-17
A	EP 0 490 631 A (HARRIS STEVEN J ;MCKERVEY MICHAEL ANTHONY (GB); SVEHLA GYULA (IE);) 17 June 1992 (1992-06-17) page 4, line 14 -page 5, line 16	1-7

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 April 2002	02/05/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax (+31–70) 340–3016	O'Sullivan, P

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